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(54) Title: ENDOGENOUS LIGANDS FOR CDR4 OF T-CELL RECEPTOR β-CHAINS AND GENES ENCODING THE SAME

(57) Abstract

The invention provides purified polypeptides corresponding to endogenous ligands and active fragments thereof that specifically bind to the CDR4 of at least one T-cell receptor β-chain. Binding agents, such as antibodies and nucleic acid probes, are also provided as well as isolated nucleic acids encoding the polypeptides. The invention further relates to methods of using these compounds for diagnostic and therapeutic purposes.

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ENDOGENOUS LIGANDS FOR CDR4 OF T-CELL RECEPTOR ß-CHAINS AND GENES ENCODING THE SAME

BACKGROUND OF THE INVENTION

This application relates to T cell immunity and more specifically to the isolation of complementary DNA molecules (cDNAs) encoding endogenous ligands for the fourth complementarity determining region (CDR4) of the T cell receptor (TCR) beta chain.

Currently, only two classes of molecules are known to be CDR4 ligands, and neither of these is encoded by genes that are truly endogenous to mammals. These two classes of molecules are the minor lymphocyte stimulatory (Mls) antigens of mice and certain bacterial exotoxins. These molecules exert profound effects on the T cell component of the immune system.

Binding of these ligands to CDR4 of the TCR beta chain (ß-chain), in the presence of a class II major histocompatibility complex (MHC) molecule-bearing antigen presenting cell, leads to the activation of those T cells, irrespective of their inherent specificity for a particular peptide antigen-MHC complex. Thus, the ligands are collectively known as "superantigens." The consequences of activation by these superantigens vary, depending on the temporal circumstances. During intra-thymic development of T cells, such activation can promote the deletion of superantigen reactive T cells and lead to their absence in the mature, peripheral repertoire. Similarly, peripheral activation of T cells by a superantigen often triggers immunoregulatory mechanisms that mediate deletion of the activated cells, such that their post-activation levels are diminished. These processes are said to "shape" the T cell repertoire; that is, the relative proportions of T cells bearing different ß-chain specificities are altered. This shaping correspondingly influences the host's ability to

mount conventional T cell responses against peptide antigens complexed with MHC molecules. An important role for superantigens in transplantation is also indicated. Binding of allogenic superantigen to CDR4 results in activation of host T cells that mediate the rejection of the allogenic lymphocyte grafts. Further, peripheral activation of T cells by superantigen can lead to the stimulation of T cells which should otherwise be tolerant to self antigens, resulting in autoimmune disease.

Given the important influences that have been demonstrated for the CDR4 ligands studied to date (e.g., superantigens), a need exists to identify endogenous CDR4 ligands. Such endogenous CDR4 ligands may have a major role in the development and maintenance of a stable, healthy T cell repertoire. Correspondingly, therapeutic regimens which modulate the activity of endogenous CDR4 ligands are anticipated to represent important new avenues for the amelioration of T cell pathogenesis. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention relates to the discovery of endogenous CDR4 ligands in humans. Accordingly, the invention provides in one aspect purified polypeptides that are specifically reactive with CDR4 of a TCR ß-chain. The polypeptides can be a purified form of an endogenous ligand or fragments thereof that contain the CDR4 binding region.

The invention further provides binding agents that are reactive with the endogenous ligand for the CDR4 of a TCR ß-chain, such as antibodies and active fragments having the CDR4 binding region. The binding agents can also be nucleic acid probes that specifically hybridize to nucleic acids encoding the endogenous ligand.

Other compounds of the present invention include conjugates and fusion proteins containing a component having a CDR4 binding region and a diagnostic or therapeutic agent. A conjugate is formed by attaching a binding agent to the diagnostic or therapeutic agent, while a fusion protein contains a first amino acid sequence encoding a binding region and a second amino acid sequence encoding a diagnostic or therapeutic agent made by recombinant or synthetic methods.

Purified or isolated nucleic acids encoding the polypeptide of the invention are also provided. The nucleic acids can be DNA, cDNA or RNA, such as mRNA. The invention further includes vectors containing the nucleic acids and additionally host cells capable of expressing the purified polypeptides.

The invention also relates to methods of detecting the presence of endogenous ligands for CDR4 of a TCR ß-chain. Detection of an endogenous ligand can be accomplished by *in vitro* or *in vivo* means. In the *in vitro* diagnostic methods, a sample from a subject is contacted with a binding agent to detect binding of any ligand in the sample to the binding agent. For *in vivo* imaging, the binding agent coupled to an imaging agent can be administered to a subject.

Methods of treating a subject having a pathology associated with the endogenous ligand are also provided in which the binding of the endogenous ligand to the CDR4 of a TCR ß-chain is inhibited. In these methods, either the CDR4 binding site of the endogenous CDR4 ligands or the CDR4 of the TCR can be blocked. Alternatively, the inhibition can be accomplished by blocking the transcription of the endogenous ligands. Various T-cell mediated pathologies can be treated using these methods, such as an autoimmune disease.

The invention in another aspect relates to kits useful in carrying out these methods. The kits can contain either the binding agents or the purified polypeptides and ancillary reagents useful for the diagnostic or therapeutic methods of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The invention generally relates to the discovery of endogenous ligands for the CDR4 of TCR ß-chains. CDR4 ligands are molecules that bind to the fourth complementarity determining region of the T cell receptor beta chain.

This discovery was based in part on the knowledge that two classes of prototypic CDR4 ligands are found in nature. Both classes exert profound effects on T cell immunity in mammalian hosts, yet neither of these two classes of molecules is encoded by genes endogenous to mammals. The mechanisms by which the known CDR4 ligands influence T cell function, as well as the magnitude of that influence, strongly suggests that endogenous CDR4 ligands exist and that they, too, serve an important role in determining the nature of the T cell immune response of an individual. Thus, identification of the endogenous CDR4 ligands represents an important advance in understanding and therapeutically manipulating T cell immunity.

Currently, only two classes of molecules are known to be CDR4 ligands: the Mls antigens in mice and certain bacterial exotoxins. These two classes of molecules exert profound effects on T cell function and thus, collectively, have come to be known as "superantigens." These known superantigens are described, for example, in Immunol. Reviews vol. 107 (1989).

Superantigens are distinguished from conventional

T cell antigens by the mechanisms through which they stimulate T cells and also by the magnitude of the T cell response which they induce. Conventional T cell antigens require internalization and processing by antigen presenting cells (APCs) prior to antigen-recognition by T cells. After internalization of the antigen by an APC, it is degraded into a large number of peptides, some of which have affinity for major histocompatibility complex (MHC) molecules. Those peptides bind to the MHC molecule and appear on the surface of the APC. T cells that are programmed to dock at MHC molecules do so, and those that possess T cell antigen receptors that are complementary to the MHC-peptide complex become activated. For conventional antigens, this activation occurs at a frequency of approximately one in one-hundred thousand T cells. The portions of the TCR that are involved in this recognition are believed to be the complementarity determining regions (CDRs) of the alpha and beta chains. Particularly important are CDRs 2 and 3 of both the alpha and beta subunits.

In contrast, superantigens do not require processing in order to stimulate a T cell response. Addition of a soluble environmental superantigen, such as a bacterial exotoxin (e.g., staphylococcal enterotoxin B) to a mixture of T cells and antigen presenting cells results in vigorous T cell proliferation, independent of APC-processing of the exotoxin molecule. Rather, the exotoxin binds to the 4th CDR of the beta chain of the TCR and also to the MHC molecule on the APC. This crosslinking provides the energy for T cell activation that is normally provided by the non-covalent interactions between the TCR and peptide antigen. In fact, during exotoxin-induced T cell activation, the peptide which is present in the cleft of the MHC molecule is irrelevant. All that is necessary for exotoxin stimulation of T cells is the presence of an APC bearing an MHC molecule and a reactive TCR ß-chain

variable region on the T cell. Different exotoxins bind to different beta chain variable regions and, thus, stimulate different populations of T cells (Marrack and Kappler, Science 248:705-711 (1990)). In addition, a particular exotoxin often will bind to more than one class of beta chain variable region and, thus, may stimulate all T cells bearing those reactive beta chains. For example, SEB will stimulate human T cells bearing Vßs 3, 12, 14, 15, 17 and 20. Collectively, T cells bearing these ß-chain variable regions may represent as much as 5% to 20% of the total T cell repertoire. Thus, exposure to this molecule will stimulate a very large fraction of T cells, relative to the one in one-hundred thousand stimulated by conventional antigen. Hence the name "superantigen."

Mls antigens also have been functionally defined as superantigens. Mls antigens are germline encoded molecules that induce T cell proliferative responses among different, but MHC-identical, strains of mice. Normally, the major T cell response against non-self cells or tissues is mediated through the recognition of MHC molecules. However, in MHC-identical mice other molecules can stimulate T cells. These other molecules have been termed minor lymphocyte stimulatory antigens or Mls antigens. When lymphocytes from a mouse that possesses a particular Mls allele (stimulators) are cultured with lymphocytes from a mouse without that allele (responders), the responder T cells proliferate vigorously. This proliferation is induced by the binding of the Mls antigen to the CDR4 region of multiple ß-chain variable regions on the surface of the responder T cells. The induced T cell proliferation is not unlike that induced by the bacterial exotoxins.

The importance of superantigens in T cell immunity is well documented. Foremost are the observations that superantigens can shape the peripheral T cell repertoire. Animals that possess a particular Mls allele

are devoid, in the periphery, of T cells bearing the ß-chain variable regions that can be stimulated by that Mls allele. This absence occurs because the Mls antigen is expressed during T cell maturation in the thymus, and those T cells that respond to the Mls antigen are deleted. When these processes are prevented by neonatal thymectomy, certain V beta-bearing T cells that are deleted in normal animals of that strain appear in the periphery of thymectomized animals, concomitant with autoimmune pathologies in multiple organs. Mls-mediated thymic deletion, thus, parallels the processes of tolerance induction which are responsible for eliminating T cells that react in a conventional fashion with self antigens. The rules are apparently the same: T cells that are activated above a certain threshold in the thymus, whether by conventional self antigen and MHC or by self superantigen and MHC, are eliminated and fail to exit from the thymus to populate the peripheral lymphoid organs. This notion has been confirmed by experiments in which bacterial exotoxins have been injected intrathymically into neonatal mice with the resulting deletion of T cells bearing the beta chain variable regions which are known to be responsive to the injected toxin.

Genetic and functional studies of Mls antigens originally suggested that they were endogenous and led to the hypothesis that all mammals would possess endogenous superantigens, with an important role in shaping the peripheral T cell repertoire. However, Mls antigens were recently shown to be encoded by the 3′ long terminal repeat of mouse mammary tumor virus (MMTV). MMTVs are retroviruses that at some point in murine evolution integrated into the germline and since have been transmitted as endogenous genes. However, in the strictest sense these are exogenous genes, of viral rather than mouse origin. Thus, enthusiasm for finding endogenous superantigens in other mammals waned among most

investigators. However, there are still reasons to suspect that true endogenous superantigens exist.

Evolutionary arguments can be made for the existence of endogenous superantigens and for their interaction with CDR4 of the TCR beta chain. It is common that environmental pathogens will use, as their receptors for entry into host cells, host proteins having their own inherent biological function. This common mechanism has evolved to insure that the pathogen's port of entry into the host cell is not altered by mutational events. Were a structural protein the pathogen's receptor, it is likely that the structural host protein could absorb numerous mutational "hits" which would alter its amino acid sequence without dramatically affecting its function. Thus, these mutations would be tolerated by the host since the protein's function would not be diminished, yet the ability of the structural protein to serve as a receptor for the pathogenic agent could be eliminated. In contrast, mutations in host molecules that have their own inherent biological function are not well tolerated. Mutations that diminish the function of the molecule will usually lead to the elimination of that mutated form of the molecule from the individual and thus from the gene pool at large. Therefore, many pathogenic agents have evolved such that they use, as receptors, host molecules of functional relevance. In this way, the selective pressures of the host insure that its port of entry will always be open for the pathogen. The fact that both viral and bacterial products have evolved mechanisms for binding to CDR4 of the beta chain indicates that CDR4 is a functionally relevant site on this molecule.

This hypothesis is supported by the lack of CDR4 sequence variation from individual to individual. Analysis of CDR4 amino acid sequences of particular V ß polypeptides indicates that these sequences are conserved. For example,

the CDR4 sequence of Vß1 polypeptides is the same in all individuals of the same species. The same is true for Vß2, Vß3 and so on. This conservation indicates that alterations in these sequences are not readily tolerated, consistent with an important functional role for this site.

The functional relevance of CDR4 is also analogous to other regions of hypervariability in the immune system. Like CDRs 1 and 2 of TCRs and immunoglobulin molecules, CDR4 is a germline encoded region of the ß-chain whose amino acid sequence varies from one ß-chain variable region to the next. CDRs 3 of TCR and immunoglobulin molecules, while not germline encoded, also show great variation from one TCR or immunoglobulin molecule to the next. MHC molecules also contain regions of germline encoded hypervariability. All these regions of variability in immune molecules are of functional importance. They serve as contact sites for each other and/or an antigen, and collectively determine the vast diversity that is characteristic of the immune system. Thus, CDR4 is ostensibly no different in these respects from other, well-characterized hypervariable regions of immune molecules.

While it might be argued that the variability in CDR4, like that in CDRs 1, 2 and 3 of the TCR, is involved in the recognition of many different MHC-peptide antigen complexes, the results of studies relating to CDR4 suggests that it is not similar to the other CDRs in this respect. Structural models of the TCR based on the X-ray crystal structure of immunoglobulin and MHC class I, indicate that CDR4 lies on a lateral face of the ß-chain, away from the distal end of the receptor which is involved in the recognition of MHC and antigen. These data suggest there is another functional relevance to the variability in CDR4 that is unrelated to antigen recognition. One hypothesis consistent with this notion is that there are endogenous

molecules that interact with CDR4 of the ß-chain and thereby influence T cell function. This hypothesis is likely the reason that bacterial exotoxins and Mls antigens exert such profound effects on T cell function in that they mimic a natural process that is important in basic T cell biology.

The cDNAs encoding endogenous, human CDR4 ligands have been isolated. These cDNAs were cloned using an anti-idiotypic serum raised against a monoclonal antibody that is known to bind to CDR4 of human Vß6.7. This antibody, a mouse monoclonal designated OT145, was injected into rabbits to produce anti-idiotypic sera that contain, in part, anti-idiotypic antibodies which are the internal image of the CDR4 combining site of OT145. Such internal image antibodies resemble CDR4 of Vß6.7 and, thus, are capable of binding to the endogenous ligand that normally associates with CDR4. These antibodies were used to screen an expression cDNA library made from human tonsil and seven antibody-reactive clones were isolated. These seven clones have been found to be remarkably similar by molecular characterization, though some differences have been seen. Given the known effects of other CDR4 ligands, such as superantigens, on T cell immunity, it is anticipated that the isolation of genes encoding endogenous CDR4 ligands represents a key advancement in further understanding human T cell immunology and in the development of new immunotherapeutic strategies.

Accordingly, the invention is directed to purified polypeptides that are specifically reactive with the CDR4 of a TCR ß-chain. The polypeptides can be used as diagnostic or therapeutic reagents, or as immunogens to produce the antibodies or their active fragments that specifically bind to the endogenous ligands of the present invention. The polypeptide can also be used to purify such antibodies or active fragments. The antibodies and their

active fragments can, in turn, be used to produce anti-idiotype antibodies that mimic the endogenous ligands. Polypeptides that recognize the CDR4 of a Vß6.7 TCR are characterized by their specific reactivity with the MH2 antibody described in Example II.

As used herein, the term "purified" is used interchangeably with "isolated" to indicate that a molecule or compound that it is substantially free of contaminants normally associated with a native or natural environment.

In addition, the terms "specifically binds," "selectively binds" and "specific reactivity" mean that a molecule binds or hybridizes to another molecule or related group of molecules, but does not substantially react with other types of molecules. For example, the purified polypeptide of the present invention specifically binds to one or more TCR ß-chains having homologous CDR4s.

The purified polypeptides of the present invention can be one of the endogenous ligands for a TCR ß-chain that has been substantially purified from a naturally-occurring source. The ligand can be purified by any means known in the art, including, for example, by affinity purification with antibodies having specific reactivity with the ligand.

A purified polypeptide can also be a fragment of one of the endogenous ligands that contains a binding region which specifically binds to CDR4. Such fragments can be obtained by any means known in the art, including the degradation of the endogenous ligands into smaller fragments with chemical reagents or with enzymes known to those skilled in the art.

A purified polypeptide of the present invention

can also be produced by well known recombinant methods as described, for example, in Maniatis et al., Molecular Cloning: A Laboratory Manual 2d ed. (Cold Springs Harbor Laboratory 1989), which is incorporated herein by reference. Alternatively, the polypeptides can be synthesized by means well known to those in the art.

Relatedly, the invention also provides isolated nucleic acids encoding the endogenous CDR4 ligands or active fragments containing the CDR4 binding region. The nucleic acids can be in the form of DNA, cDNA or RNA, particularly mRNA. They can also be chemically synthesized by methods known in the art, including an automated nucleic acid synthesizer.

The invention further provides binding agents. As used herein, the term "binding agent" refers to any molecule that specifically binds or hybridizes with the endogenous ligands of the present invention or genes encoding the CDR4 ligands. Such binding agents can include, for example, antibodies, active fragments of such antibodies and nucleic acid probes. Active fragments of the antibodies can also include peptides containing a binding region that specifically binds to the endogenous ligands. Accordingly, active fragments of such antibodies include, for example, Fab and Fab'_2 fragments, as well as other peptides having specificity for the endogenous CDR4 ligands.

The binding agents of the invention can be produced by any method known in the art. For example, polyclonal and monoclonal antibodies as well as various active fragments thereof can be produced by well known methods, as described for example in Harlow and Lane, Antibodies: A Laboratory Manual (1988), incorporated herein by reference. Such agents may also be produced synthetically by methods known in the art or by recombinant

methods described, for example, in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Springs Harbor Laboratory 1989), which is incorporated herein by reference.

Nucleic acid probes that hybridize to the nucleic acid sequence associated with the expression of the endogenous ligand can be prepared from the cloned sequences or by synthesizing oligonucleotides that hybridize only with the homologous sequence under reasonably stringent conditions. The probes can be labeled with labels and markers according to methods known in the art and used to detect DNA or mRNA. In addition, the probes can be used to hybridize to a nucleic acid sequence of the genes encoding the endogenous CDR4 ligands to prevent or enhance the transcription of the ligands.

The isolated nucleic acids of the present invention can be inserted into vectors that express the desired nucleic acid sequence. The term "vector" includes vectors that are capable of expressing nucleic acid sequences operably linked to sequences capable of effecting their expression. These nucleic acid sequences can encode the purified polypeptides or binding agents of the present invention. Numerous cloning vectors are known in the art. Thus, the selection of an appropriate cloning vector is a matter of choice. In general, useful vectors for recombinant DNA techniques are often plasmids, which refer to circular double stranded DNA loops. As used herein, "plasmid" and "vector" may be used interchangeably as the plasmid is the most common form of a vector. However, the invention is intended to include other forms of expression vectors that serve equivalent functions.

Suitable host cells containing the nucleic acid sequences encoding the purified polypeptides or binding agents of the invention are also provided. Host cells can be transformed with a vector and used to express the

desired recombinant or fusion protein. Methods of recombinant expression in a variety of host cells, such as mammalian, yeast, insect or bacterial cells, are widely known, including those described in Maniatis et al., Molecular Cloning: A Laboratory Manual 2d ed. (Cold Springs Harbor Laboratory 1989), which is incorporated herein by reference.

The invention further provides methods for determining the presence of an endogenous CDR4 ligand in a subject. The term "subject" refers any vertebrate, including humans, capable of having a T cell mediated pathology or clonal T cell replication. The diagnostic methods can be accomplished by *in vitro* or *in vivo* imaging means.

For the *in vitro* methods, a sample obtained from a subject is contacted with a binding agent and the binding of the agent with the endogenous CDR4 ligand or its gene is detected. The endogenous ligand or gene can be detected by any suitable means for detecting proteins or nucleic acids known in the art. Immunological and hybridization techniques for assaying proteins and nucleic acids, respectively, are generally well known in the art, such as those descibed in Maniatis *et al.*, supra.

Immunological procedures useful for the *in vitro* detection of the endogenous ligand in a sample such as blood, urine, serum, tissues or tissue extracts, or other bodily fluids, include immunoassays that employ a detectable antibody or an active fragment having specific reactivity with the ligand. Such immunoassays include, for example, ELISA, agglutination assays, flow cytometry, serum diagnostic assays, immunohistochemical staining procedures which are well known in the art.

The binding agent, such as an antibody, can be

made detectable by various means well known in the art. For example, a detectable marker can be directly or indirectly attached to the binding agent. Useful markers include, for example, radionuclides, enzymes, fluorogens, chromogens and chemiluminescent labels.

For the *in vivo* detection methods, a detectable binding agent is administered to a subject and the binding of the agent to the endogenous CDR4 ligand is detected by imaging techniques known in the art. Suitable imaging agents are known and include, for example, gamma-emitting radionuclides such as ^{111}In, ^{99m}Tc, ^{51}Cr and the like, as well as paramagnetic metal ions, which are described in U.S. Patent No. 4,647,447, incorporated herein by reference. The radionuclides permit the imaging of tissues or organs by gamma scintillation photometry, positron emission tomography, single photon emission computed tomography and gamma camera whole body imaging, while paramagnetic metal ions permit visualization by magnetic resonance imaging.

Conjugates and fusion proteins containing a component having specific reactivity with the endogenous CDR4 ligand and a diagnostic agent are useful for the *in vitro* and *in vivo* detection methods. The conjugates can be produced by attaching a binding agent, such as an antibody or an active fragment, to a diagnostic agent by means well known in the art. Fusion proteins are obtained by fusing a nucleic acid encoding a binding agent with a nucleic acid encoding a diagnostic (or therapeutic) agent and expressing a protein encoded by the fused nucleic acids. The term "diagnostic agent" refers to any agent capable of being detected either with or without the aid of instrumentation. When used for the *in vivo* methods, a diagnostic agent is also referred to as an imaging agent.

As discussed above, CDR4 ligands such as the Mls antigen and bacterial exotoxins are known to be associated

with more than one Vßs, which in turn are known to mediate various T cell pathologies. The pathologies include, for example, autoimmune diseases such as rheumatoid arthritis, and multiple sclerosis, for example. Therefore, the present methods can be used for the prevention and treatment of such pathologies.

The methods of treating a subject having a pathology mediated by an endogenous CDR4 ligand can be accomplished by inhibiting the binding of the CDR4 ligand to the CDR4 of a TCR ß-chain. The binding of the endogenous CDR4 ligand to CDR4 can inhibited by various means. For example, a binding agent having specific reactivity with an endogenous CDR4 ligand at the CDR4 binding site of the ligand can be used to block the ligand/CDR4 binding. Alternatively, the transcription of the endogenous CDR4 ligand can be block by various means, including, for example, using a nucleic acid probe that attaches to the gene encoding the ligand at a site that blocks the transcription of the ligand or at a site that inhibits the transcription of a functional ligand.

The ligand/CDR4 binding can also be inhibited by interfering with the CDR4 of a TCR ß-chain. For example, an antibody fragment or other small peptide having a CDR4 binding region can be used to compete with the endogenous CDR4 ligand for binding to the CDR4.

Alternatively, the methods of preventing or treating a T cell mediated pathology can be accomplished by administering to the subject a binding agent having specific reactivity with an endogenous CDR4 ligand attached to a therapeutic agent. In this regard, the binding agent acts as a delivery vehicle for the therapeutic agent or for the diagnostic agent in the *in vivo* imaging methods. The conjugates and fusion proteins described above can be used in the treatment and diagnostic methods as well.

Suitable therapeutic agents can be cytotoxic or cytostatic agents such as various drugs, radionuclides or toxins known to those skilled in the art. Useful drugs include, for example, the vinca alkaloids, daunomycins, mitomycins, bleomycins and colchicines. Beta-emitting radionuclides such as ^{90}Y and ^{111}In are useful as therapeutic agents. Toxins suitable as therapeutic agents include ricin, podophyophyllotoxins, the trichothecenes, pseudomonas endotoxin and the like.

Therapeutic agents can also be vaccines for the prevention or treatment of a pathology mediated by an endogenous CDR4 ligand. The vaccine can be, for example, an active fragment of a TCR as described in PCT publication no. WO 91/13632, published on September 19, 1991, which is incorporated herein by reference, a non-infectious viral antigen such as HIV or other known vaccines.

The binding agents, conjugates or fusion proteins of the invention can be administered to the subject by means known in the art, including parenteral means such as intravascular, intraperitoneal, subcutaneous or intramuscular injection. For parenteral administration, these compounds can be administered in an admixture with a pharmaceutically acceptable carrier. Such carriers are well known and include, for example, aqueous solutions such as bicarbonate buffers, phosphate buffers, Ringer's solution and physiological saline.

The pharmaceutical compositions useful for imaging and therapy can be prepared by methods known in the art, including the simple mixing of the reagents. Those skilled in the art will know that the choice of the pharmaceutical carrier and the appropriate preparation of the composition will depend on the intended use and mode of administration.

The dosage regimen for the *in vivo* diagnostic and therapeutic methods depends on a variety of factors, including the age, weight, and medical condition of the patient, as well as the type of disorder, the severity of the condition, the route of administration and the diagnostic or therapeutic agent used. A skilled physician or veterinarian can readily determine and prescribe the effective amount of the compound or pharmaceutical composition required. Because the binding agent can localize the diagnostic or therapeutic agent, much lower doses of these agents can be used compared to conventional doses.

For administration of the purified polypeptide of the present invention, the dose is preferably about 1-250 μg, and more preferably about 50-100 μg.

Finally, kits useful for carrying out the methods of the invention are also provided. The kits contain either the purified polypeptide or a binding agent and an ancillary reagent. Such ancillary reagents include diagnostic or therapeutic agents, signal detection systems, buffers, stabilizers, pharmaceutically acceptable carriers or other materials that are known to be included in such kits.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Production of anti-idiotypic sera to OT145

The monoclonal antibody OT 145 was obtained from T Cell Sciences (Cambridge, MA). The antibody, in PBS, was emulsified with an equal volume of complete Freund's adjuvant and 50 μgs were injected into each of two New Zealand White rabbits at multiple subcutaneous sites. One month later, rabbits were boosted with an additional 50 μg of OT 145 emulsified with an equal volume of incomplete Freund's adjuvant. Ten days later, rabbits were bled through the ear vein and sera tested for reactivity with OT 145. Pre-immune sera were obtained from each rabbit prior to immunization.

EXAMPLE II

Characterization of anti-idiotypic OT145 sera

Reactivity of anti-OT145 sera was tested by ELISA. Wells of microtiter plates were coated overnight at 4 degrees with 50 μl of an OT 145 solution containing 5-10 μg of antibody in 0.1 M bicarbonate buffer, pH 8.3. Unbound antibody was removed and wells were blocked with 100 μl PBS containing 2% bovine serum albumin (PBS-BSA). Rabbit antisera were serially diluted in PBS containing 0.1% BSA and 0.05% Tween-20. Fifty μl of each dilution were added to triplicate wells. After one hour, unbound antibody was removed with three washes with PBS containing 0.05% Tween-20 (PBS-Tween). 50 μl of alkaline-phosphatase conjugated goat-anti-rabbit IgG (KPL, Gaithersburg, MD) (1:2500 dilution in PBS-BSA and Tween-20) were added and incubated for one hour at 37 degrees. Wells were washed seven times with PBS-Tween, once with PBS and once with dH_2O. The alkaline phosphatase substrate, para-nitrophenyl

phosphate, was added and reactivity monitored by absorbance at 410 nm. Each of the rabbit sera contained significant antibody titers to OT145 (Table IA). The anti-idiotypic antisera are designated as MH1 and MH2 from rabbits 1 and 2, respectively.

TABLE I

A. Reactivity of anti-OT145 sera vs OT145

Dilution	MH1	MH2	MH1 Adsorbed	MH2 Adsorbed
1:500	0.892	0.829	0.607	0.542
1:1000	0.809	0.741	0.542	0.494
1:2000	0.722	0.523	0.501	0.416
1:4000	0.559	0.374	0.415	0.260
1:8000	0.395	0.222	0.300	0.170

B. Reactivity of anti-OT145 vs mIgG

Dilution	MH1	MH2	MH1 Adsorbed	MH2 Adsorbed
1:225	0.997	0.886	0.051	0.033
1:675	0.827	0.679	0.020	0.010
1:2025	0.556	0.324	0.033	0.026

EXAMPLE III

Adsorption of anti-idiotypic OT145 sera

Immune sera were adsorbed against normal mouse IgG (mIgG) to remove rabbit antibodies reactive with conserved mouse IgG determinants. Normal mouse serum was adjusted to 45% saturation with ammonium sulfate and the IgG fraction collected by centrifugation at 10,000 x g. After dialysis, IgG was coupled to cyanogen bromide (CNBr)-activated Sepharose)Pharmacia, Piscataway, NJ) at a ration

of 1 mg of antibody per ml of gel. Rabbit were diluted 1:1 with PBS and applied to the mIgG column, previously equilibrated in PBS-FBS. Flow-thru fractions were collected and those containing unbound rabbit IgG were pooled and tested by ELISA. As shown in Table IB, adsorption of these sera completely eliminated reactivity with mIgG. The OT145 titer was reduced to approximately 10% of the original anti-OT145 activity.

EXAMPLE IV

Screening the bacteriophage expression library with absorbed sera

Immune and pre-immune sera were adsorbed against a lysate of E. coli proteins to minimize background reactivity in subsequent screenings of the bacteriophage library. A lambda gtll lysogen was produced in E. coli Y1089 and the synthesis of beta-galactosidase was induced with 10 mM iso-propyl-thio-galactoside (IPTG) (Sigma, St. Louis, MO) for one hour. Bacteria were collected by centrifugation at 4000 x g, resuspended in 50 mM Tris HCl, pH 8.0, 10 mm ethylenediaminetetraacetic acid and 0.1% phenyl-methyl-sulfonyl-fluoride (TEP buffer) and flash frozen in liquid nitrogen. The extract was thawed, sonicated briefly and dialyzed. This extract of E. coli proteins was coupled to CNBr- Sepharose and used to adsorb rabbit sera as above.

A cDNA library made from human tonsil was obtained from The American Type Culture Collection (Rockville, MD). This library was constructed in the bacteriophage lambda cloning vector, gtll, to permit expression of the cDNA inserts and screening with antibody probes.

E. coli Y1090 r- (Promega, Madison, WI) were infected with 550,000 plaque forming units (Pfus) of the

phage library and plated in top agarose. Plates were incubated 3 hours at 42 degrees and were overlaid with dry nitrocellulose filters that previously had been saturated with 10 mM IPTG. After an additional 3 hour incubation at 37 degrees, the first filter was removed and duplicate filters were incubated an additional 3 hours. Filters were blocked at room temperature overnight in PBS containing 0.05% Tween-20, 10% nonfat milk and 5% normal goat serum. Each of the filter sets was incubated one hour at room temperature with one of the two rabbit sera, diluted 1:1000 in PBS containing 0.05% Tween-20 (PBS-Tween). After three washes with PBS-Tween, alkaline phosphatase conjugated goat-anti-rabbit IgG was added (1:2500 dilution in PBS-Tween) and incubated for an hour at room temperature. Filters were washed three times with PBS-Tween, once with PBS and once with dH_2O. BCIP/NBT substrate (KPL, Gaithersburg, MD) was added and antibody-positive phage clones were identified.

Of the 550,000 phage plaques screened, seven antibody-positive phage were obtained with antiserum from rabbit number two and none with antiserum from rabbit number one. Positive phage were picked and cloned by three cycles of plaque purification. These clones were designated 2.1, 2.2, 2.5, 2.6, 2.7, 2.8, and 2.9. The intensity with which the antibodies bound these plaques varied. Based on these variations, the phage clones could be segregated into groups. Clones 2.1, 2.2, 2.6, and 2.9 produced signals of approximately equal intensity. Clones 2.5 and 2.7 were bound by the rabbit antibodies to equivalent degrees, yet much more strongly (estimated, 2 to 3 fold) than clones 2.1, 2.2, 2.6 and 2.9. Clone 2.8 produced an intermediate signal, binding more strongly than clones 2.1, 2.2, 2.6, 2.9 but more weakly than clones 2.5 and 2.7.

EXAMPLE V

Characterization of phage cDNA inserts

The relative sizes of the seven cDNA inserts were determined by polymerase chain reaction (PCR)- catalyzed amplification. Minipreps of phage DNA were isolated from plate lysates and PCR amplified using primers that flank the unique Eco R1 cloning site of lambda gt11. The sequences of these primers are: gt11 sense strand primer; 5' GGT GGC GAC GAC TCC TGG 3' (SEQ ID NO: 1), and; gt11 anti-sense strand primer; 5' CCA GAC CAA CTG GTA ATG 3' (SEQ ID NO: 2). The products of these PCR reactions were electrophoresed in agarose gels. The fragments amplified from five of the phage clones were found to be approximately 580 base pairs in length, indicating an insert size of approximately 500 bp (clones 2.1, 2.2, 2.5, 2.6 and 2.9). One of the phage clones had an insert of approximately 4 kb (clone 2.7) and one had an insert of approximately 1.5 kb (clone 2.8).

The observation that five of the phage clones possessed inserts of the same size suggested that they were similar if not identical. PCR-amplified inserts from clones 2.1, 2.2, 2.5, 2.6 and 2.9 were analyzed for restriction fragment length polymorphism (RFLP) with seventeen different restriction enzymes. The enzymes tested were: Ava I, Cla I, Sal I, Hind III, Eco R1, Sau 3A, Mbo I, Taq I, Nhe I, Bgl II, Dde I, Rsa I, Hae III, Alu I, Hinf I, Pst I and Apa I. None of these enzymes revealed differences among the five cDNA isolates, suggesting that these five isolates represent the same gene or a group of closely related genes.

To further characterize these clones, partial DNA sequences have been determined. Phage DNAs were PCR-amplified and the amplified products were digested with Eco

R1 and cloned into the Eco R1 site of the plasmid vector Bluescript KS (Stratagene, La Jolla, CA). Plasmid clones containing the 500 bp inserts of page clones 2.1 and 2.5 were obtained, as well as plasmid clones containing the 1.5 kb insert of phage clone 2.8. Digestion of the 4kb insert from phage clone 2.7 cleaved at Eco R1 sites in the cDNA as well as at the Eco R1 sites of gt11 into which the cDNA had been cloned. Thus, an intact 4kb fragment was not cloned into Bluescript. Rather, a seven hundred base pair fragment was obtained. Partial DNA sequence has been obtained for each of these 4 clones using sequencing primers that anneal to the T3 and T7 RNA polymerase promoters present in Bluescript. The sequences of these primers are: T3, 5′ ATT AAC CCT CAC TAA AG 3′ (SEQ ID NO: 3); and, T7, 5′ AAT ACG ACT CAC TAT AG 3′ (SEQ ID NO: 4).

At the five prime end of the cDNAs from phage clones 2.1, 2.5, a stretch of approximately 200 bp was found to be substantially similar, if not identical. At the three prime end of these cDNAs, a stretch of approximately 200 bp also was found to be substantially similar, if not identical. The five prime end of the 700 bp fragment derived from clone 2.7 also showed strong similarity, if not identity to the 2.1 and 2.5 sequences. Five prime and three prime sequences from clone 2.8 showed only slight similarity to the other three clones.

The majority of the sequence data obtained to date is considered partial, since little of it has yet been confirmed by sequencing both DNA strands. However, 89 base pairs of sequence, obtained from clone 2.5, have been verified on both strands with no inconsistencies. The sequence of this 89 bp stretch is:

GGACTTAAAACAATTCATGAAAGTGGACCTTTAAAAGCTTGTCAGAGTTGCACAAAT CTAACTGGTATTTTGTTTTTGTTTTTAGGAGG (SEQ ID NO: 5).

The DNA database, Genbank (release 70), was searched for sequences with significant homology to this 89 bp sequence using the program FASTA. None of the Genbank sequences contained this exact region of 89 bp, indicating that these genes are new and have not been identified previously.

The assignment of 5 prime and 3 prime ends of these cDNAs described above derives from the localization of the poly A tail of the original cDNAs from clones 2.1 and 2.5. This information was used to verify that the cDNAs were present in gt11 in an orientation that would direct the synthesis of the bona fide protein produced in the tonsil from which the library was made. A reverse orientation of these cDNAs in gt11 would mean that the protein produced by the recombinant phage and detected by our antibody would derive from the non-coding strand of the cDNA. Little biological significance could be attributed to such reactivity. A PCR primer, corresponding to a sequence found at the three prime end of clone 2.1 and 2.5 CDNAS, was synthesized. The sequence of this primer is: 5′ GAG GTT TAA CAT GAA ATG 3′ (SEQ ID NO: 6). This primer, an anti-sense strand primer, was used in conjunction with the sense strand gt11 primer that resides 5 prime of the Eco R1 cloning site in gt11. Only clones which contain the CDNA insert in the correct orientation relative to the beta-galactosidase promoter of gt11 (which is what facilitates the expression of the CDNAS) will give rise to PCR product using this primer pair. All of the phage clones with 500 base pair inserts (i.e., 2.1, 2.2, 2.5, 2.6 and 2.9) produced PCR products of approximately 480 bp (approximately 440 bp derived from the insert). This indicates that these clones all have the CDNA inserted into gt11 in the proper orientation. Further, it supports the notion that these are similar or identical genes, since the binding site for this 3 prime, antisense primer is present

in the same position in all of these clones. Clones 2.7 and 2.8 also produced PCR products when amplified with this primer pair. Clone 2.7 produced a 870 bp product upon amplification and phage clone 2.8 produced a product of approximately 780 bp. These two cDNAs, therefore, are also in the proper orientation in gt11. The presence of the binding site for the 3 prime primer in these two clones further argues their relatedness to clones 2.1, 2.2, 2.5, 2.6 and 2.9.

Although the invention has been described with reference to the disclosed embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: COLORADO STATE UNIVERSITY RESEARCH FOUNDATION

(ii) TITLE OF INVENTION: ENDOGENOUS LIGANDS FOR CDR4 OF T-CELL RECEPTOR BETA-CHAINS AND GENES ENCODING THE SAME

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CAMPBELL AND FLORES
(B) STREET: 4370 LA JOLLA VILLAGE DRIVE, SUITE 700
(C) CITY: SAN DIEGO
(D) STATE: CALIFORNIA
(E) COUNTRY: UNITED STATES
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 07-JUN-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: KONSKI, ANTOINETTER F.
(B) REGISTRATION NUMBER: 34,202
(C) REFERENCE/DOCKET NUMBER: FP-IM 9650

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-535-9001
(B) TELEFAX: 619-535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTGGCGACG ACTCCTGG 18

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCAGACCAAC TGGTAATG 18

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATTAACCCTC ACTAAAG 17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATACGACTC ACTATAG 17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGACTTAAAA CAATTCATGA AAGTGGACCT TTAAAAGCTT GTCAGAGTTG CACAAATCTA 60

ACTGGTATTT TGTTTTTGTT TTTAGGAGG 89

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGGTTTAAC ATGAAATG 18

We claim:

1. A purified polypeptide specifically reactive with CDR4 of a T-cell receptor ß-chain.

2. The purified polypeptide of claim 1, wherein said polypeptide has specific reactivity with antibody MH2.

3. The purified polypeptide of claim 1, wherein said ß-chain is Vß6.7.

4. A binding agent having specificity for an endogenous ligand for CDR4 of a T-cell receptor ß chain or for a nucleic acid encoding said ligand.

5. The binding agent of claim 4, wherein said binding agent has specificity for said endogenous ligand.

6. The binding agent of claim 5, wherein said binding agent is an antibody or an active fragment thereof.

7. The binding agent of claim 4, wherein said binding agent specifically hybridizes to said nucleic acid.

8. The binding agent of claim 7, wherein said binding agent is a nucleic acid probe.

9. A conjugate comprising a binding agent attached to a diagnostic or therapeutic agent, said binding agent having specific reactivity with an endogenous ligand for CDR4 of a T-cell receptor ß-chain.

10. The conjugate of claim 9, wherein said binding agent is an antibody or active fragment thereof.

11. The conjugate of claim 9, wherein said diagnostic or therapeutic agent is radiolabelled.

12. The conjugate of claim 9, wherein said therapeutic agent is a cytotoxic or cytostatic agent.

13. The conjugate of claim 12, wherein said therapeutic agent is a toxin.

14. The conjugate of claim 9, wherein said therapeutic agent is a vaccine.

15. The conjugate of claim 14, wherein said vaccine comprises an active fragment of a T cell receptor.

16. The conjugate of claim 14, wherein said vaccine comprises a non-infectious viral antigen.

17. The conjugate of claim 16, wherein said viral antigen is HIV.

18. A fusion protein comprising a first amino acid sequence encoding the binding agent of claim 5 and a second amino acid sequence encoding a diagnostic or therapeutic agent.

19. The fusion protein of claim 18, wherein said first amino acid sequence encodes an antibody or active fragment thereof.

20. The fusion protein of claim 19, wherein said second amino acid sequence encodes a vaccine, a toxin, a detectable marker or a radiolabelled agent.

21. A purified nucleic acid encoding the polypeptide of claim 1.

22. The purified nucleic acid of claim 21, wherein said nucleic acid is DNA.

23. The purified nucleic acid of claim 21, wherein said nucleic acid is RNA.

24. The purified nucleic acid of claim 21, wherein said nucleic acid is cDNA.

25. A vector containing the nucleic acid of claim 21.

26. A host cell containing the vector of claim 25.

27. A method for determining the presence of an endogenous ligand for CDR4 of a T-cell receptor ß-chain in a subject, comprising:

(a) contacting a sample obtained from said subject with a binding agent of claim 4; and

(b) detecting the binding of said agent to said endogenous ligand, in which binding indicates the presence of said endogenous ligand in the subject.

28. The method of claim 27, wherein said binding agent is a nucleic acid probe.

29. The method of claim 27, wherein said binding agent is an antibody or an active fragment thereof.

30. A method for determining the presence of an endogenous ligand for CDR4 of a T-cell receptor ß-chain in a subject, comprising:

(a) administering the binding agent of claim 4 to said subject; and

(b) detecting the binding of said agent to said ligand, wherein binding indicates the presence of said ligand in the subject.

31. The method of claim 30, wherein said binding agent is an antibody or an active fragment thereof.

32. The method of claim 30, wherein said binding agent is attached to a diagnostic agent in a conjugate.

33. The method of claim 30, wherein said binding agent is fused to a diagnostic agent in a fusion protein.

34. A method of treating a subject having a pathology mediated by an endogenous ligand for CDR4 of a T cell receptor ß-chain, comprising inhibiting the binding of said endogenous ligand to said CDR4.

35. The method of claim 34, wherein said inhibition is accomplished by blocking transcription of said endogenous ligand.

36. The method of claim 34, wherein said inhibition is accomplished by binding said ligand with a binding agent having specificity for said endogenous ligand at the CDR4 binding region of said ligand.

37. The method of claim 36, wherein said binding agent is an antibody or an active fragment thereof.

38. The method of claim 34, wherein said inhibition is accomplished by binding said CDR4 with an

active fragment of an antibody having specific reactivity with said CDR4 to modulate T cell immunity.

39. The method of claim 34, wherein said pathology is an autoimmune disease.

40. A kit comprising the binding agent of claim 4 and an ancillary reagent.

41. The kit of claim 40, wherein said binding agent is an antibody, an active fragment thereof or a nucleic acid probe.

42. A kit comprising the purified polypeptide of claim 1 and an ancillary reagent.

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/US 93/05417

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 G01N33/566 C12Q1/70 A61K47/48 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGY vol. 147, no. 10 , 15 November 1991 , BALTIMORE US pages 3441 - 3444 Y.PRASHAR ET AL. 'A monoclonal antibody (OT145)specific for the T cellantigen receptor v 6.7a allele detects an epitope related to a proposed superantigen-binding site' see the whole document ---	1-20,27, 29-33, 40-42
X	SCIENCE. vol. 244 , 19 May 1989 , LANCASTER, PA US pages 811 - 813 J.KAPPLER ET AL. 'V -specific stimulation of human T cells by Staphylococcal toxins' see the whole document --- -/--	1,2,9,12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 November 1993	03. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	CUPIDO, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna l Applicaaon No
PCT/US 93/05417

C.(Conanuaaon) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citaaon of document, with indicaaon, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 23 , 1 December 1991 , WASHINGTON US pages 10921 - 10925 M.D.HOWELL ET AL. 'Limited T-cell receptor -chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis' see table 5	4-9,14, 15
X	WO,A,90 11294 (THE IMMUNE RESPONSE CORPORATION) 4 October 1990 see the whole document	4-9,14, 15
E	WO,A,93 12814 (THE IMMUNE RESPONSE CORPORATION) 8 July 1993 see figure 1; examples XIII,XIV	4-9,14, 15

2

Form PCT/ISA/210 (conanuaaon of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

:national application No.
PCT/US93/05417

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 34-39 are directed ot a mehtod of treatment of the human/animal body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant s protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/US 93/05417

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9011294	04-10-90	AU-A-	5356790	22-10-90
		EP-A-	0463101	02-01-92
		JP-T-	4506512	12-11-92
WO-A-9312814	08-07-93	AU-B-	3418893	28-07-93